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## Microbiological and clinical assessment of the abutment and non-abutment teeth of partial removable denture wearers

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### ABSTRACT

**Objective:** The aim of this study was assessing the changes in both clinical and microbiological parameters of healthy individuals after rehabilitation with removable partial denture (RPD).

**Design:** 11 women received unilateral or bilateral free-end saddle RPD in the mandibular arch. Clinical and microbiological parameters of abutment, non-abutment, and antagonist teeth were assessed at baseline (RPD installation) and after 7, 30, 90, and 180 days of function. The Checkerboard DNA–DNA hybridization technique was used to identify and quantify up to 43 different microbial species from subgingival biofilm samples. Probing depth, gingival recession, and bleeding on probing were also investigated over time.

**Results:** The total and individual microbial genome counts were shown significantly increased after 180 days with no significant differences between abutment, non-abutment, or antagonist teeth. *Streptococcus* spp., *Aggregatibacter actinomycetemcomitans*, and other species associated to periodontitis (*Peptostreptococcus anaerobius*, *Prevotella nigrescens*, and *Tannerella forsythia*), as well as opportunistic *Candida* spp., were recovered in moderate counts. Abutment teeth presented higher values of gingival recession when compared with non-abutment or antagonist teeth, irrespectively time of sampling ( $p < 0.05$ ). No significant differences were found between groups regarding bleeding on probing or probing depth over time.

**Conclusions:** Overall, the microbial counts significantly increased after 6 months of denture loading for both abutment and non-abutment teeth with no significant differences regarding the microbial profile over time. Bleeding on probing and probing depth showed no significant difference between groups over time whereas gingival recession increased in the abutment teeth.

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### 1. Introduction

Removable Partial Denture (RPD) is a fast and cost-effective treatment indicated for the replacement of missing teeth in partially edentulous individuals presenting large or small free-way space (Bohnenkamp, 2014; Jones & Turkyilmaz, 2010). Some patients are unable to afford rehabilitation with fixed prostheses or dental implants due to anatomic, systemic, or economic reasons, therefore RPD may be considered a non-invasive and relatively cheap treatment with a predictable long-term success, achieving appropriate esthetics, increasing the masticatory efficiency, and

improving phonetics (Nassani, Tarakji, Baroudi, & Sakka, 2013; Bohnenkamp, 2014). However, they can represent a risk for the remaining teeth – mainly abutment teeth – depending on the prosthesis design and health of the supporting periodontal tissues (Preshaw et al., 2011). For this reason, RPD are not indicated in individuals with tooth mobility higher than 1 mm (Mojan et al., 2016)

Studies have been shown that RPD has an increased risk of biofilm formation on teeth surfaces closing to the clasps or attachments. In addition, this condition was shown enhanced in the abutment teeth (Dula, Ahmedi, Lila-krasniqi, & Shala, 2015; Vacaru et al., 2003). In distal-extension prosthesis (Kennedy class I and II), abutment teeth receiving clasps are more susceptible to mechanical loading, which could cause damage to the periodontium resulting in tooth mobility (Drake & Beck, 1993; Jorge et al., 2012). Many studies have shown that RPDs have been increased plaque, gingival, and calculus index with potential risk to develop

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gingivitis and root caries; however, there is not clear evidence regarding their impact on the initiation of periodontitis (Bergman, 1987; Drake & Beck, 1993; Petridis & Hempton, 2001; Zlatarić, Celebić, & Valentić-Peruzović, 2002; Do Amaral et al., 2010).

There is a consensus in the applied literature that an intense oral hygiene control must be held in RPD wearers in order to avoid or minimize biofilm formation. The biofilm control of patients is essential for the long-term success of the treatment (Benson & Spolsky, 1979; Isidor & Budtz-Jørgensen, 1990; Khan, Ahamed, Alhadlaq, Musarrat, & Al-Khedhairy, 2013). Longitudinal studies have been shown that regular biofilm control and denture hygiene can improve the periodontal health of individuals with RPD (Akaltan & Kaynak, 2005; Zlatarić et al., 2002). However, most of the studies evaluating the periodontal status of abutment and non-abutment teeth of RPD wearers are restricted to report the potential changes occurred in the clinical parameters (plaque and calculus index, probing depth, bleeding on probing, and tooth mobility) with no information on the microbial profile. Considering that oral cavity may harbor over than 500 different bacterial species and that complex oral microbial ecosystem may be changed by RPD's wearers, the investigation of this microbial profile may provide new insights on the periodontal status over time. The composition of the oral bacterial community in these patients has not been fully understood. DNA or RNA-specific probes have not only largely redefined oral microbial taxonomy (including fastidious or non-cultivable species), but they have now permitted the rapid analysis of the complex microflora composition in a large number of samples (Zhu et al., 2012). The Checkerboard DNA–DNA Hybridization is a technique that gives a simultaneous quantitative analysis of up to 28 biofilm samples tested against up to 45 microbial species.

Thus, the purpose of this clinical study was assessing the changes in both clinical and microbiological parameters of healthy individuals after rehabilitation with removable partial denture (RPD). Checkerboard DNA–DNA hybridization was used to identify and quantify up to 43 different microbial species colonizing abutment and non-abutment teeth; the microbiological findings were related with a set of clinical outcomes (probing depth, gingival recession, and bleeding on probing) at 5 different time points up to 6 months of functional loading. The null hypothesis tested in this study is that there is a significant difference in the microbial profile from abutment or non-abutment teeth over time.

## 2. Material and methods

### 2.1. Participants selection

The participants enrolled in this study were selected among partially edentulous individuals referred to the Prosthodontics Clinic of School of Dentistry of São Paulo University. There were 11 women with a mean age of 53.3 ( $\pm 13.0$ ) years and a ranging from 33 to 71 years. This longitudinal study was approved by the local Ethics Committee on Research from USP (CAAE 0077.0.138.00-12) and all the experiments were undertaken with the informed and written consent of each subject according to ethical principles. Potential participants were selected if they had total bilateral or unilateral distal free-end space in the mandibular arch (Kennedy Class I or II) and their upper maxillaries were completely dentate. Exclusion criteria included diabetes, cardiopathy, hypertension, pregnancy, habits of drinking, current smokers, any using of other RPDs, dental caries, gingival inflammation, tooth mobility, recent extractions, pocket depth greater than 3 mm, periodontal or antibiotic therapy in the previous 3 months, and any systemic condition which could influence the course of periodontal status.

### 2.2. Experimental design and data collection

RPD was prepared respecting individual clinical conditions, remaining teeth, and according to the biomechanical principles. Three patients were treated with bilateral distal-extension and eight were treated with unilateral distal-extension dentures. A cobalt–chromium framework was used in the RPD structures. The retainers included T-bar clasps for abutment teeth and circumferential clasps for non-abutment teeth. All the denture structures were connected by a lingual bar.

Before clinical rehabilitation, all the selected participants received oral hygiene instructions followed by supra and subgingival manual scaling/root planing and ultrasonic debridement. All the prostheses were installed after 7 days of the professional cleaning. Abutment teeth used as direct or indirect retainer for the RPD were set as experimental group, while the non-abutment teeth in the same jaw were set as control group. Additionally, the antagonist teeth from maxilla were also set as another control group. Microbiological sampling and clinical data collection were conducted at baseline—during RPD installation ( $T_0$ ) and after 7-days ( $T_1$ ), 30-days ( $T_2$ ), 90-days ( $T_3$ ), and 180-days ( $T_4$ ) of functional loading. A complete periodontal examination using a manual periodontal probe (Hu-Friedy; Chicago, IL, USA) was conducted to record the clinical parameters (probing depth, gingival recession, and bleeding on probing). Each tooth (mesial, medial, and distal aspects) was probed twice to reduce the potential error in probing angulation and the final measurement for probing depth and gingival recession was a mean of the 2 evaluations. At the same time, the subgingival biofilm samples from periodontal sulci of tested teeth were collected with sterile paper points (Dentsply, Dentsply/Maillefer, Ballaigues, Switzerland). Each subgingival sample was a pool of 6 paper points exposed for 30 s into the periodontal sulcus, 3 in the buccal and 3 in the palatal/lingual aspects, respectively in mesial, medial, and distal positions. Individual samples were placed in microtubes containing 150  $\mu$ L of TE Buffer (10 mM Tris–HCl, 1 mM EDTA, pH = 7.6) followed by addition of 150  $\mu$ L 0.5 M NaOH. Samples were stored at  $-20^\circ\text{C}$  until laboratorial processing.

### 2.3. Microbiological analysis

The identification and quantification of microbial species recovered from the investigated teeth were performed by Checkerboard DNA–DNA hybridization technique according to Do Nascimento et al. (2010). Thirty-eight bacterial species, including putative periodontal pathogens (*Aggregatibacter actinomycetemcomitans* serotypes *a* and *b*, *Bacteroides fragilis*, *Capnocytophaga gingivalis*, *Campylobacter rectus*, *Escherichia coli*, *Eikenella corrodens*, *Enterococcus faecalis*, *Fusobacterium nucleatum*, *Fusobacterium periodonticum*, *Klebsiella pneumoniae*, *Lactobacillus casei*, *Mycoplasma salivarium*, *Neisseria mucosa*, *Porphyromonas aeruginosa*, *Peptostreptococcus anaerobius*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella melaninogenica*, *Parvimonas micra*, *Prevotella nigrescens*, *Pseudomonas putida*, *Staphylococcus aureus*, *Streptococcus constellatus*, *Streptococcus gordonii*, *Streptococcus mitis*, *Solobacterium moorei*, *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus parasanguinis*, *Staphylococcus pasteuri*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Streptococcus sobrinus*, *Treponema denticola*, *Tannerella forsythia* and *Veillonella parvula*) and 5 *Candida* species (*C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. krusei* and *C. tropicalis*) frequently found harbouring the oral microbiota of healthy and diseased individuals were investigated. Briefly, the DNA samples from each tooth (abutment, non-abutment, and antagonist) were denatured

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